

2017

An incipient invasion of brown anole lizards (*Anolis sagrei*) into their own native range in the Cayman Islands: a case of cryptic back-introduction

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Citation/Publisher Attribution

Kolbe, J.J., Wegener, J.E., Stuart, Y.E. et al. *Biol Invasions* (2017) 19: 1989. <https://doi.org/10.1007/s10530-017-1432-2>
Available at: <https://doi.org/10.1007/s10530-017-1432-2>

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2 Cayman Islands - a case of cryptic back-introduction

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15 16 ABSTRACT

17 Human-mediated dispersal has reshaped distribution patterns and biogeographic
18 relationships for many taxa. Long-distance and over-water dispersal were historically rare events
19 for most species, now human activities can move organisms quickly over long distances to new
20 places. An eventual consequence of human activities is the potential reintroduction of
21 individuals from an invasive population back into their native range; a dimension of biological
22 invasion termed "cryptic back-introduction." We investigated whether this phenomenon was
23 occurring in the Cayman Islands where brown anole lizards (*Anolis sagrei*) with red dewlaps

(i.e., throat fans), either native to Little Cayman or invasive on Grand Cayman, have been found on Cayman Brac where the native *A. sagrei* have yellow dewlaps. Our analysis of microsatellite data shows strong population-genetic structure among the three Cayman Islands, but also evidence for non-equilibrium. We found some instances of intermediate multilocus genotypes (3-9% of individuals), particularly between Grand Cayman and Cayman Brac. Furthermore, analysis of dewlap reflectance data misclassified some males sampled on Cayman Brac as having red dewlaps similar to lizards from Grand Cayman and Little Cayman. Lastly, one individual from Cayman Brac had an intermediate microsatellite genotype, a red dewlap, and a mtDNA haplotype from Grand Cayman. This mismatch among genetic and phenotypic data documents that invasive *A. sagrei* from Grand Cayman are interbreeding with native *A. sagrei* on Cayman Brac. To our knowledge, this is the first evidence of "cryptic back-introduction." Although we clearly demonstrate this phenomenon is occurring in the Cayman Islands, assessing its frequency there and prevalence in other systems may prove difficult due to the need for genetic data. Cryptic back-introductions may eventually provide some insight into how the invasion process changes a lineage as compared to how evolution shaped the lineage in its native range.

KEYWORDS

admixture; dewlap; microsatellites; nuclear-mitochondrial mismatch; reproductive isolation

44 INTRODUCTION

45 In this era of widespread human-mediated dispersal, geographic distance is no longer a
46 barrier to movement for many species. Exotic species introductions have altered fundamental
47 biogeographic patterns such as species-area and species-isolation relationships (MacArthur &
48 Wilson 1963; Sax et al. 2005). For example, colonization by exotic *Anolis* lizards in the
49 Caribbean has altered pre-invasion biogeographic patterns (Losos & Schluter 2000), effectively
50 reducing geographic isolation among islands and flattening the negative species-isolation
51 relationship for anoles (Helmus et al. 2014). Long-distance colonization events due to human
52 activity can bring together evolutionarily distinct lineages that in some instances have been
53 separated for millions of years. Novel interactions among these lineages could reshape existing
54 ecological communities and alter patterns of species diversity (e.g., Stuart et al. 2014; Liu et al.
55 2014). Furthermore, hybridization or admixture between introduced and native taxa (e.g.,
56 Fitzpatrick & Shaffer 2007) might compromise the genetic distinctiveness of taxa, including
57 endemics, unique phenotypes, and evolutionarily significant units (Moritz 1994). A previously
58 undocumented dimension of biological invasion could occur when invasive populations from
59 outside of their native range are reintroduced into native-range populations. Guo (2005)
60 proposed the term "cryptic back-introduction" for this phenomenon, but to our knowledge it has
61 never been demonstrated. Here we explore whether this phenomenon is occurring in the brown
62 anole lizard (*Anolis sagrei*) and consider the implications of this type of invasion for native
63 populations.

64 *Anolis sagrei* is a good natural colonizer, reaching many islands and mainland areas in
65 the Caribbean by overwater dispersal (Williams 1969). During its ~6.5 million year evolutionary
66 history (R.G. Reynolds, pers. comm.), this species colonized all of Cuba, most islands in the

Bahamas, the Atlantic coast of Mexico and Belize, and Swan Island (Schwartz & Henderson 1991). Approximately 2.5 million years ago this species also colonized Cayman Brac and Little Cayman (R.G. Reynolds, pers. comm.), two small and relatively remote islands in the western Caribbean. Subsequently, *A. sagrei* differentiated into the yellow-dewlapped endemic subspecies *A. s. luteosignifer* on Cayman Brac and the red-dewlapped *A. s. sagrei* on Little Cayman (Schwartz & Henderson 1991); the dewlap is an extendable throat fan used for communication in anoles (Losos 2009). However, *A. sagrei* failed to colonize the third of the Cayman Islands, Grand Cayman, which is located ~100 km to the southwest of the other Cayman Islands. That is, until the early 1980s when a red-dewlapped form of *A. sagrei* became established on Grand Cayman via a human-mediated introduction on cargo shipments from established non-native populations in South Florida (Minton & Minton 1984; Kolbe et al. 2004; Fig. 1), thereby accomplishing a long-distance dispersal event that failed to occur naturally. Since this time, inter-island supply shipments by air and sea within the Caymans—primarily from the capital and largest port on Grand Cayman (196 km²) to the much smaller Cayman Brac (~38 km²) and Little Cayman (<10 km²)—could have transported invasive and native *A. sagrei* among this trio of islands. Indeed, in 2010, a single *A. sagrei* individual with a red dewlap was observed on Cayman Brac (T. Sanger, pers. comm.), suggesting an introduction of invasive *A. sagrei* from Grand Cayman or native *A. s. sagrei* from Little Cayman.

In this study, we surveyed *A. sagrei* populations across Cayman Brac looking for red-dewlapped lizards to determine whether invasive *A. sagrei* from Grand Cayman have in fact invaded Cayman Brac. We also collected lizards on Grand Cayman and Little Cayman to discriminate between these two potential sources of red-dewlapped *A. sagrei*. For lizards from all three islands, we quantified dewlap phenotypes using spectrophotometric methods, measured

structural habitat use (i.e., perch height and diameter) and body size (i.e., snout-vent length [SVL], and mass), and genotyped 10 nuclear microsatellite loci. For individuals with intermediate multilocus genotypes or a genotype that did not match their island, we sequenced mtDNA haplotypes (ND2) to test for nuclear-mitochondrial mismatches. Using these data, we evaluated whether invasive *A. sagrei* from Grand Cayman have been introduced to native populations on Cayman Brac, and if so, whether invasive lizards have interbred with native lizards.

METHODS

We sampled lizards in the Cayman Islands in January 2011, focusing the majority of our effort on Cayman Brac due to the previous observation of a red-dewlapped *A. sagrei* there (Fig. 1 & Table S1). On all three islands, for each undisturbed lizard encountered, we measured perch height (cm) and perch diameter (cm), two key aspects of the structural habitat niche in anoles (Losos 2009). We also measured two aspects of body size, SVL (mm) and mass (g), for all lizards captured. We caught only males because females do not have dewlaps (see below). We used analysis of variance (ANOVA) to test for differences among islands in lizard structural habitat use and body size using JMP (SAS 2014). Perch diameter values were log-transformed to achieve a normal distribution.

To quantify dewlap color, we measured reflectance spectra from 300 to 700 nm in wavelength (i.e., visible plus ultraviolet light) using an Ocean Optics USB4000 spectrophotometer with a pulsed Xenon light source (PX-2, Ocean Optics), calibrated regularly against a white reflectance standard (Labsphere, Inc.). We measured reflectance at nine points distributed across the dewlap by pressing the dewlap against a black background with the

reflectance probe containing a black, 45°-angled tip used to prevent specular glare. The Ocean Optics software was set to a boxcar width of five and an integration time of ten milliseconds. We averaged ten scans to generate spectra for each point on the dewlap.

We used the R-package *pavo* (Maia 2013) to manipulate spectrophotometric data. Briefly, we used the functions *getspec* to import our spectra within the 300-700 nm wavelength window, *aggspec* to generate a mean spectrum for each individual, and *procspec* to bin those spectra into 10-nm windows. With the values from these 10-nm bins, we used the base package R-function *prcomp* to conduct a principal component analysis (PCA) in R (R Core Team 2014). We tested for differences among islands on PC axes representing dewlap reflectance using ANOVA and Tukey's Honestly Significant Difference (HSD) *post hoc* tests using JMP (SAS 2014). We then used discriminant function analysis (DFA) to predict group membership based on dewlap reflectance data from PC axes using JMP (SAS 2014). Lizards from Grand Cayman and Little Cayman were combined *a priori* into a red dewlap group and Cayman Brac lizards were in the yellow dewlap group.

After dewlap data were collected, we removed ~15 mm of tail tissue for genetic analysis. The tissues were preserved in 100% ethanol and stored at -20°C. We extracted genomic DNA following a standard protocol using the AutoGenprep 965 machine (Autogen, Inc.) in the Bauer Core Facility at Harvard University. We amplified 10 microsatellite loci using PCR (Bardeleben et al. 2004; Wordley et al. 2011) and fluorescently labeled primers following standard protocols at the University of Rhode Island. Samples were genotyped at the DNA Analysis Facility at Yale University. Markers for all samples were analyzed with the software GeneMapper® v4.1 and visually inspected for accuracy. For a subset of individuals, we sequenced the mtDNA marker ND2. Sequences were assembled and aligned in Geneious Pro v5.6.3 (Kearse et al. 2012). Data

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4 136 were combined with previously published microsatellite genotypes (Kolbe et al. 2008) and
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6 137 mtDNA (ND2) sequences for the Cayman Islands (Kolbe et al. 2004, 2007).
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9 138 Using these multilocus microsatellite data, we estimated standard population genetic
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11 139 statistics for diversity within islands and tested for Hardy-Weinberg equilibrium for each locus
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14 140 on each island. To evaluate population differentiation among the three islands under the
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16 141 assumption of Hardy-Weinberg equilibrium, we calculated pairwise F_{ST} values using GenAlEx
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18 142 (Peakall & Smouse 2012) and conducted a Bayesian cluster analysis using STRUCTURE
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20 143 (Pritchard et al. 2000). We also assessed population differentiation among islands with methods
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23 144 that do not rely on assumptions of equilibrium, including principal coordinates analysis (PCoA)
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25 145 using GenAlEx and discriminant analysis of principal components (DAPC) using the *adegenet*
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27 146 package in R (Jombart & Ahmed 2012). To determine the relationships among ND2 haplotypes,
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29 147 we constructed a maximum likelihood phylogeny using RAxML (Stamatakis et al. 2008;
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31 148 Silvestro & Michalak 2012). The final alignment consisted 540 bp of ND2 for 63 individuals,
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34 149 including samples from the Cayman Islands, Cuba, South Florida and *A. bremerei* as the
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36 150 outgroup (Kolbe et al. 2007). We used GTR+G, the best fitting available model for nucleotide
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39 151 substitution based on Modeltest 3.7 (Posada & Crandall 1998). For the ML search, we used the
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41 152 rapid bootstrapping algorithm with 1000 non-parametric replicates.
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47 154 RESULTS

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50 155 We sampled a total of 280 male *A. sagrei* from 18 sites on Cayman Brac (n=195), two
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52 156 sites on Little Cayman (n=26), and six sites on Grand Cayman (n=59) (Fig. 1 & Table S1). We
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54 157 found no differences among islands in structural habitat use (perch height: $F_{2,201}=1.32$, $P=0.27$
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57 158 and log-perch diameter: $F_{2,173}=0.32$, $P=0.73$) or body size (SVL: $F_{2,254}=0.02$, $P=0.98$ and mass:
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4 159 $F_{2,241}=2.38$, $P=0.10$; Table S2). When comparing PC axes, dewlap reflectance showed strong
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6 160 differentiation between yellow-dewlapped lizards on Cayman Brac and the red-dewlapped
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8 161 lizards on the other two islands (Fig. 2 & Table 1A). DFA based on PC axes 1-4, which
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10 162 represented 98% of variation, misclassified six lizards (CB008, CB026, CB046, CB107, CB170,
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12 163 CB178), all of which were sampled on Cayman Brac but classified as having a red dewlap (Fig.
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14 164 2). This supports our field observations of seemingly red-dewlapped lizards occurring on
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16 165 Cayman Brac (Fig. 3) and suggests the introduction of invasive *A. sagrei* from Grand Cayman or
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18 166 native *A. s. sagrei* from Little Cayman. One lizard from Little Cayman was misclassified as
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20 167 being from Cayman Brac (LC015).

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22 168 Cayman Brac had higher allelic diversity compared to the other two islands and Hardy-
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24 169 Weinberg equilibrium was rejected for most loci on this island (Table 2); both results are
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26 170 consistent with an ongoing influx of alleles to Cayman Brac. F_{ST} values showed greater
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28 171 differentiation between Grand Cayman and the other two islands ($F_{ST}=0.209-0.235$) compared to
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30 172 that between Cayman Brac and Little Cayman ($F_{ST}=0.161$; Table S3). Moreover, STRUCTURE
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32 173 identified two genetic clusters corresponding to 1) Grand Cayman and 2) Cayman Brac and
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34 174 Little Cayman (Fig. S1). However, rejection of HWE for half the loci across islands (Table 2)
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36 175 violates the assumption of equilibrium for F_{ST} and STRUCTURE analyses. Avoiding this
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38 176 assumption, comparison of PCo axes from multilocus microsatellite genotypes and genetic
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40 177 differentiation based on DAPC analysis showed similarly strong differentiation among islands
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42 178 (Figs. 4 & S2; Table 1B), particularly Grand Cayman compared to the other two islands.

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44 179 We identified genetically intermediate individuals on the PCo 1 v. PCo 2 plot (Fig. 4) as
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46 180 those outside of the 99.7% confidence ellipse (i.e., 7 of 280 individuals, or 3%; CB007, CB101,
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48 181 CB104, CB109, CB118, CB144, GC066) or the 95% confidence ellipse (i.e., 25 of 280
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4 182 individuals, or 9%). Most lizards with intermediate microsatellite genotypes were sampled on
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6 183 Cayman Brac and all but one had mtDNA haplotypes matching their island. One lizard sampled
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9 184 on Cayman Brac (CB008) had a Grand Cayman mtDNA haplotype (see phylogenetic tree in Fig.
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11 185 S3), an intermediate genotype outside 95% confidence ellipse for Cayman Brac, and a red
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14 186 dewlap misclassified in the DFA (Figs. 2-4). Another individual sampled on Cayman Brac
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16 187 (CB101) had a microsatellite genotype indistinguishable from other Little Cayman lizards, yet it
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19 188 had a Cayman Brac mtDNA haplotype and a yellow dewlap (Figs. 2 & 4). These two examples
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21 189 demonstrate that red-dewlapped lizards from both Grand Cayman and Little Cayman have been
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24 190 introduced to Cayman Brac and have subsequently interbred with endemic *A. s. luteosignifer*
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26 191 there. In addition to these definitive examples, numerous individuals show some mismatch
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29 192 among their microsatellite genotype, mtDNA haplotype, and dewlap phenotype. For instance,
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31 193 most lizards from Cayman Brac with misclassified red dewlaps had unambiguously Cayman
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33 194 Brac microsatellite genotypes and mtDNA haplotypes.
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41 197 Our genetic and phenotypic data document for the first time the reintroduction of
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43 198 individuals from an invasive population back into their native range, termed "cryptic back-
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45 199 introduction" (Guo 2005). Nuclear-mitochondrial mismatches clearly demonstrate that red-
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48 200 dewlapped *A. sagrei* from both Grand Cayman and Little Cayman have interbred with endemic,
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51 201 yellow-dewlapped *A. s. luteosignifer* on Cayman Brac. This supports human-mediated gene flow
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53 202 among islands in the Caymans, including transfer of invasive *A. sagrei* from Grand Cayman to
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55 203 native populations on Cayman Brac. Although demonstrative, the two lizards with nuclear-
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58 204 mitochondrial mismatches (< 1%) could represent merely a low incidence of invasion.
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Alternatively, when including genetic intermediates and lizards with misclassified dewlaps, 4-11% of lizards may be the product of admixture between invasive and native populations. Less clear is when and how many times *A. sagrei* has arrived on Cayman Brac over the past ~30 years since it invaded Grand Cayman, making it difficult to assess whether invasive alleles are persisting on Cayman Brac or merely being introduced repeatedly.

When invasive individuals from outside of their native range are introduced into a native population at least two outcomes are possible. First, invasive and native lineages may fail to interbreed, and therefore represent cryptic species. Given that the two species will overlap substantially in their ecological niches, interspecific interactions such as competition may be important. If the invader is favored, then the persistence of the native species may be in jeopardy. Alternatively, if the native lineage is favored in interspecific interactions, then the native range may be a sink for propagules that arrive but whose alleles do not persist. A second possibility is that the lineages interbreed, altering the genetic distinctiveness of the native populations. This occurred when introduced tiger salamanders (*Ambystoma tigrinum*) hybridized with threatened California tiger salamanders (*Ambystoma californiense*), leading to changes in fitness, population viability, and patterns of natural selection that favored some invasive alleles (Fitzpatrick & Shaffer 2007; Fitzpatrick et al. 2010). Given genetic admixture of *A. sagrei* among geographically and genetically distinct native-range lineages during its invasion of Florida (Kolbe et al. 2004, 2008), successful interbreeding among invasive and native lineages in the Caymans is not surprising. In this instance, however, interbreeding occurred despite clear differences in dewlap color, a trait used for species recognition in anoles (Losos 2009) and therefore could have served as a reproductive barrier between lineages. The genetic mixing of these lineages raises the pressing issue of how invasive *A. sagrei*, which are genetically and

phenotypically divergent from native lineages (Kolbe et al. 2004, 2007, 2014), will alter the previously isolated populations on Cayman Brac.

The very nature of cryptic back-introductions makes it difficult to identify this phenomenon based on morphology alone, requiring genetic analyses in most instances. Invaders may harbor other types of phenotypic variation, such as in behavior or physiology, relevant for invasion success. A classic example of a cryptic invasion is the replacement of native common reed (*Phragmites australis*) haplotypes in New England by a non-native haplotype over the past 100 years (Saltonstall 2002). The spread of this invasive haplotype is at least in part due to greater salinity tolerance and higher relative growth rates compared to native haplotypes (Vasquez et al. 2005), providing a mechanistic explanation for this invasion. Some aspects of phenotypic variation in invasive *A. sagrei* populations in Florida, including body size, head and limb proportions, and perhaps metabolic rate, are related to the invasion history and source populations (Kolbe et al. 2007, 2014). Other phenotypes like low-temperature tolerance and water loss rate, which follow clines in temperature and moisture in Florida, are suggestive of adaptive responses (Kolbe et al. 2012, 2014). South Florida is the source of the Grand Cayman introduction (Kolbe et al. 2004), so whether these traits or others differ between invasive Grand Cayman *A. sagrei* and native lizards on Cayman Brac remains to be seen.

Our findings show that lizards with different colored dewlaps can interbreed; however, our data are insufficient to determine confidently whether assortative mating and asymmetric introgression are occurring or if mating is random. Females of this species do not have dewlaps, thus males cannot use this trait for mate choice. Unlike other *Anolis* species that have stereotyped display patterns used during courtship (e.g., *Anolis carolinensis*; DeCourcy & Jenssen 1994; Losos 2009), male *A. sagrei* may lack courtship-specific displays on which females could base

mate selection (Scott 1984). However, dewlap color and other courtship behaviors could faithfully signal the origin or genetic identity of a male lizard, but little is known about mate selection based on intraspecific variation in anoles. Our preliminary assessment of cyto-nuclear disequilibrium in genetically and phenotypically intermediate lizards from Cayman Brac shows only one case of a lizard with a Grand Cayman mtDNA haplotype (which is maternally derived), suggesting a higher frequency of mating between red-dewlapped males from Grand Cayman or Little Cayman and females from Cayman Brac.

The evolutionary history of isolated island groups has been largely contingent upon rare, long-distance dispersal events (Gillespie & Clague 2009). Periods of isolation promote divergence that leads to reproductive isolation and eventually speciation. It took *A. sagrei* millions of years to colonize Cayman Brac and Little Cayman naturally. In contrast, after finally invading Grand Cayman via human-mediated dispersal, invasive *A. sagrei* spread via human activity to native populations on Cayman Brac within just 30 years. As human commerce in the Anthropocene decreases the "distances" among islands (Helmus et al. 2014), these types of cryptic back-introductions are expected to increase in frequency. If inter-island introductions in the Caymans continue, or even increase, we predict the Cayman Brac lineage will lose its unique genetic and phenotypic identity. Unfortunately, the cryptic nature of back-introductions makes them difficult to monitor without genetic analyses. We therefore do not yet know the frequency with which cryptic back-introductions are occurring. Interbreeding will likely blend traits, like dewlap color, that could contribute to species recognition, potentially derailing speciation and contributing to homogenization (McKinney & Lockwood 1999). Cryptic back-introductions may eventually provide some insight into whether the process of invasion changes lineages in ways that make them distinct from and potentially incompatible with conspecific native populations.

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275 ACKNOWLEDGEMENTS

276 We thank Haley Moniz, Vikram Chhatre, Brianne Neptin, Melissa Losos, Bonnie Edwards, and
277 the URI Genome and Sequencing center staff for assistance. Permits were graciously provided
278 by Matthew Cottam and the Department of Environment of the Cayman Islands. Funding was
279 provided by a Putnam Expeditionary Grant from the Museum of Comparative Zoology, and the
280 University of Rhode Island.

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Fig. 1. Map showing portions of the native and non-native ranges of the brown anole relevant to this study: A) arrows indicate routes of primary invasion from multiple native-range source populations in Cuba to the non-native range in Florida and a secondary invasion from non-native populations in south Florida to Grand Cayman; B) the arrow indicates a cryptic back-introduction of invasive brown anoles from Grand Cayman into the native range on Cayman Brac; and B & C) circles show collection localities for genetic, ecological, morphological, and dewlap data on Grand Cayman, Little Cayman, and Cayman Brac with site numbers corresponding to those listed in Table S1.

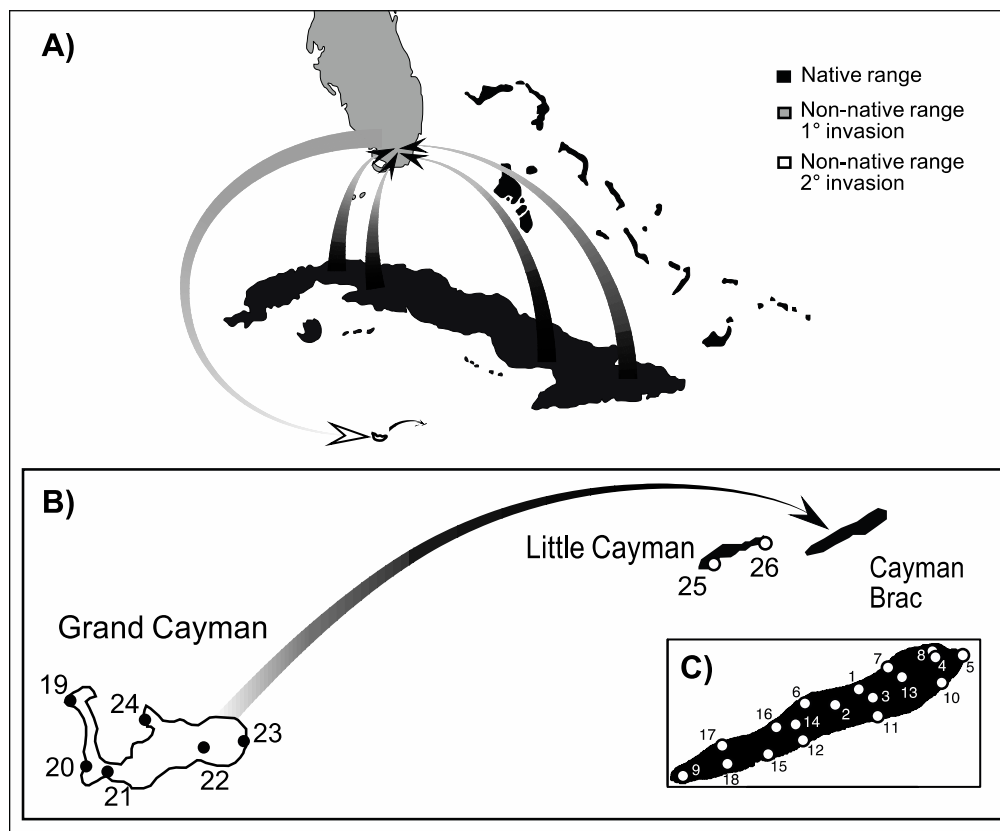


Fig. 2. Results of a PCA for dewlap reflectance using the average wavelength from the dewlap of each male lizard. Photos of lizards with their dewlaps extended represent the color variation along each PC axis.

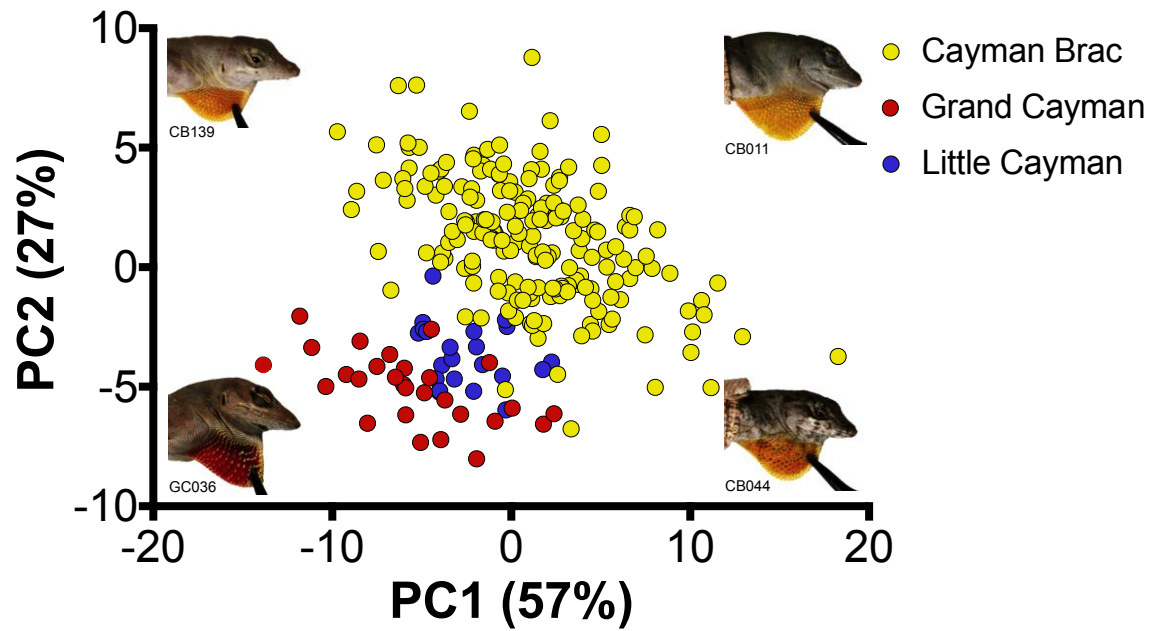


Fig. 3. Examples of dewlaps of brown anoles from A) Grand Cayman, B) Cayman Brac with misclassified dewlaps, C) Cayman Brac with intermediate genotypes, D) Cayman Brac with native genotypes, and E) Little Cayman. Genetically intermediate individuals were outside of the 95% confidence ellipse for Cayman Brac and intermediate between Cayman Brac and Grand Cayman based on the PCoA of multilocus microsatellite genotypes (see Fig. 4). Misclassified dewlaps were sampled on Cayman Brac, but grouped with red dewlaps (see Fig. 2 and Supplementary Materials for photographic methods).

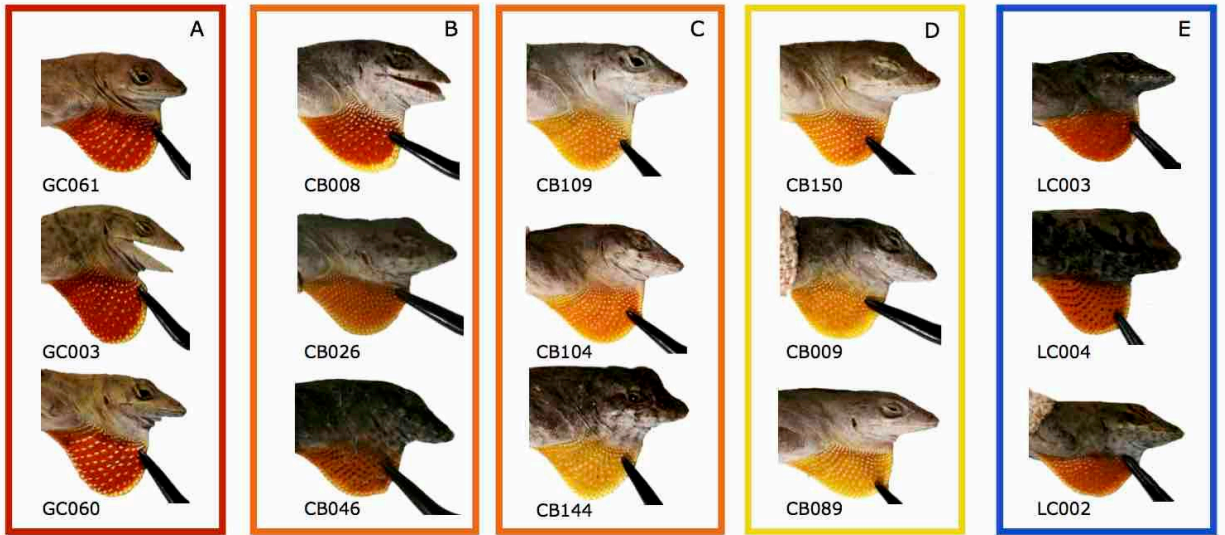


Fig. 4. Results of a PCoA using multilocus genotypes from 10 microsatellite loci. Confidence ellipses are based on 2SD (95%) and 3SD (99.7%) from the mean. Outliers beyond confidence limits are interpreted as individuals that are genetically intermediate between islands.

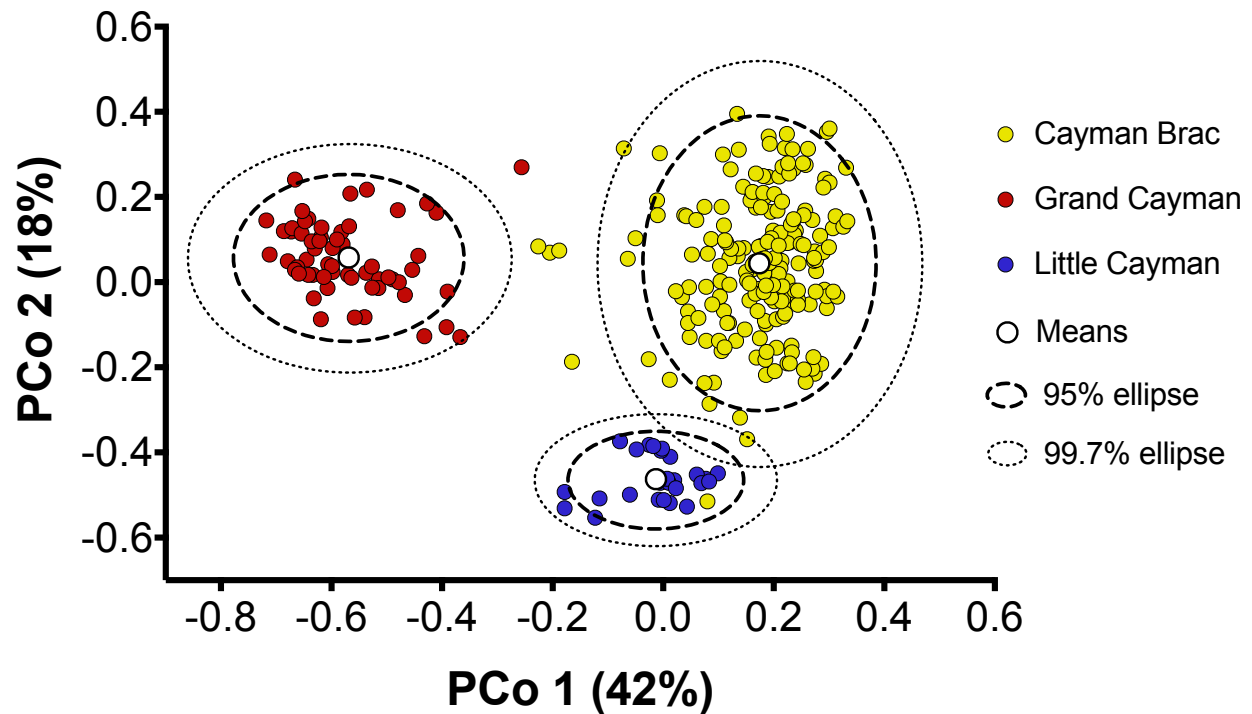


Table 1. Results of ANOVAs and Tukey's HSD post hoc tests for differences among islands in A) dewlap reflectance based on PC axes (n=244) and B) multilocus microsatellite genotypes based on PCo axes (n=280). Abbreviations are CB=Cayman Brac, LC=Little Cayman, and GC=Grand Cayman.

A)

PC axis	PC % variation	R ²	df	F	P	Tukey's HSD
PC1	57%	0.223	2,241	35.5	< 0.0001	CB>LC>GC
PC2	27%	0.475	2,241	109.2	< 0.0001	CB>LC=GC
PC3	8%	0.043	2,241	5.4	0.005	CB=LC>GC
PC4	4%	0.024	2,241	3.8	0.052	GC≥CB≥LC

B)

PCo axis	PCo % variation	R ²	df	F	P	Tukey's HSD
PCo1	42%	0.908	2,277	1363.1	< 0.0001	CB>LC>GC
PCo2	18%	0.530	2,277	156.4	< 0.0001	GC=CB>LC
PCo3	14%	0.049	2,277	7.1	0.001	GC=CB>LC
PCo4	11%	0.020	2,277	2.9	0.058	GC=CB=LC

Table 2. Summary statistics for population genetic analyses based on multilocus microsatellite genotypes sampled from the Cayman Islands. N = number of genotypes, N_A = number of alleles, N_E = effective number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = fixation index within islands, HWE = number of loci not in Hardy-Weinberg equilibrium ($P < 0.05$). Asterisk (*) indicates that four loci for the Little Cayman population were monomorphic.

Island	N	N_A	N_E	H_O	H_E	F_{IS}	HWE
Cayman Brac	195	14.1 ± 3.4	5.32 ± 1.84	0.44 ± 0.08	0.55 ± 0.10	0.22 ± 0.06	8/10 loci
Grand Cayman	59	8.0 ± 1.1	3.51 ± 0.38	0.53 ± 0.06	0.66 ± 0.06	0.18 ± 0.07	4/10 loci
Little Cayman	26	7.2 ± 2.4	4.97 ± 1.68	0.38 ± 0.12	0.47 ± 0.14	0.19 ± 0.07	1/6 loci*
Mean	88.3	9.7 ± 1.5	4.60 ± 0.82	0.45 ± 0.05	0.56 ± 0.06	0.20 ± 0.04	13/26 loci*

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4 457 SUPPLEMENTARY MATERIALS
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6 458 METHODS
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9 459 Photography
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11 460 We collected dewlap data using photographic approaches. Photographs were taken with a
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14 461 Canon Rebel XTi Digital SLR camera set to custom white balance calibrated with a WhiBal®
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16 462 reference card (Michael Tapes Design). The right side of each lizard's dewlap was extended
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19 463 against an 18% Delta 1 Gray Card (CPM, Inc.), adjacent to a ruler and a Mini ColorChecker®
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21 464 color card (x-rite, Inc.) and photographs were taken without the flash, in a dark room with the
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24 465 lizard illuminated by two 50W Halogena bulbs (Philips, Inc.). Tracing paper was placed in front
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26 466 of the bulbs to diffuse the light and reduce glare. Lizards were immobilized using self-adherent
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29 467 medical wrap and dewlaps were extended with self-closing forceps whose tips were coated with
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31 468 black rubber (Plasti Dip International) to reduce glare.
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Table S1. Collection localities and sample sizes for brown anoles in the Cayman Islands. Site IDs correspond to the numbers on the map in Fig. 1. Sample sizes used for dewlap and microsatellite analyses are provided.

Island	Site (Map ID)	GPS Degrees N	GPS Degrees W	N (Dewlap / Genetics)
Cayman Brac	L&M Superstore (1)	19.73244	-79.78450	11/11
Cayman Brac	Botanical Garden (2)	19.72380	-79.79838	12/12
Cayman Brac	Soccer Field (3)	19.72782	-79.77621	11/11
Cayman Brac	Peter's Cave Overlook (4)	19.75312	-79.74110	10/10
Cayman Brac	Lighthouse (5)	19.75124	-79.72344	10/10
Cayman Brac	R&L Plant Nursery (6)	19.72461	-79.81614	10/10
Cayman Brac	Port Authority (7)	19.74444	-79.76731	11/11
Cayman Brac	Peter's Cave Trailhead (8)	19.75355	-79.74127	11/12
Cayman Brac	Brac Reef Resort (9)	19.68459	-79.88777	11/11
Cayman Brac	South Road (east end) (10)	19.73616	-79.73581	14/14
Cayman Brac	South Road (empty lot) (11)	19.71769	-79.77339	10/10
Cayman Brac	1758 South Road (12)	19.70435	-79.81732	10/11
Cayman Brac	Bluff Road (13)	19.73918	-79.75911	11/11
Cayman Brac	Deadman's Point Trail (14)	19.71303	-79.82159	10/10
Cayman Brac	Bat Cave (15)	19.69661	-79.83772	10/10
Cayman Brac	Cayman Brac Museum (16)	19.71143	-79.83292	10/9
Cayman Brac	Billy's Supermarket (17)	19.70124	-79.86472	10/11

Cayman Brac	Rebekah's Cave (18)	19.69120	-79.86161	12/12
Grand Cayman	Cayman Turtle Farm (19)	19.38014	-81.41726	0/4
Grand Cayman	Sunset House (20)	19.28604	-81.38998	28/32
Grand Cayman	South Coast Hurley's (21)	19.28156	-81.34593	0/5
Grand Cayman	Queen Elizabeth Botanical Gardens (22)	19.31614	-81.16895	0/8
Grand Cayman	Sunnyfield Road (23)	19.32827	-81.08532	0/5
Grand Cayman	Cayman Kai (24)	19.36738	-81.27356	0/5
Little Cayman	Blossom Village (25)	19.65912	-80.08347	22/26
Little Cayman	East End (26)	19.69801	-79.97370	0/4

Table S2. Means \pm SD for measurements of body size (SVL and mass) and structural habitat use (perch height and diameter).

Island	N	SVL (mm)	N	Mass (g)	N	Perch height (cm)	N	Perch diameter (cm)
Cayman Brac	194	50.3 \pm 5.7	194	3.6 \pm 1.2	149	66.5 \pm 53.7	127	6.9 \pm 8.3
Grand Cayman	41	50.1 \pm 5.5	28	3.1 \pm 0.9	39	55.9 \pm 40.0	33	6.4 \pm 6.2
Little Cayman	22	50.3 \pm 5.5	22	3.5 \pm 1.2	16	81.4 \pm 60.2	16	6.3 \pm 3.5

Table S3. Pairwise F_{ST} values (\pm SE) comparing the three Cayman Islands with all samples for each island pooled on the off-diagonal and the mean pairwise F_{ST} values (\pm SE) for sampling sites within islands on the diagonal, only two sites were sampled on Little Cayman.

	Cayman Brac	Grand Cayman	Little Cayman
Cayman Brac	0.042 \pm 0.015		
Grand Cayman	0.209 \pm 0.019	0.071 \pm 0.028	
Little Cayman	0.161 \pm 0.023	0.235 \pm 0.019	0.118

Fig. S1. Results of the Bayesian cluster analysis using the multilocus microsatellite genotypes indicate that $K=2$ is the most likely number of genetic clusters. Populations from Cayman Brac and Little Cayman form a single genetic cluster distinct from Grand Cayman. Vertical lines represent individuals and results show few individuals with mixed ancestry between the two genetic clusters.

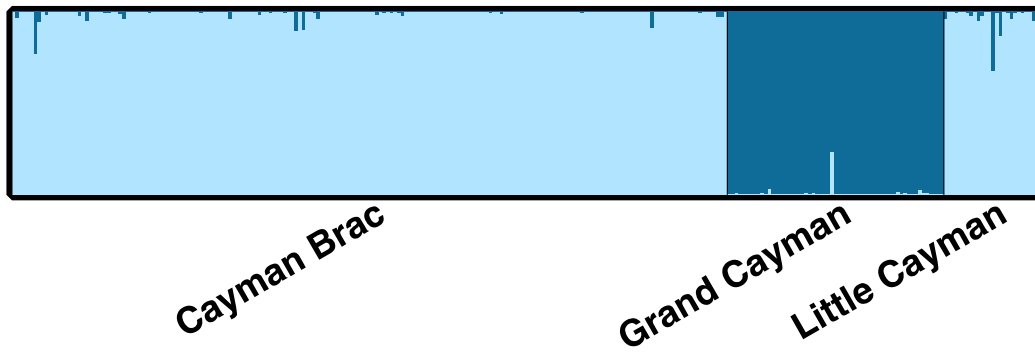


Fig. S2 - Genetic relatedness among individuals and genetic groupings on the first two PC axes from a discriminant analysis of principal components (DAPC). Results are similar to the PCoA (Fig. 4) and STRUCTURE (Fig. S1) analyses in that Cayman Brac (yellow) and Little Cayman (blue) are less differentiated (they form a single genetic cluster in the STRUCTURE analysis) than either is to Grand Cayman (red).

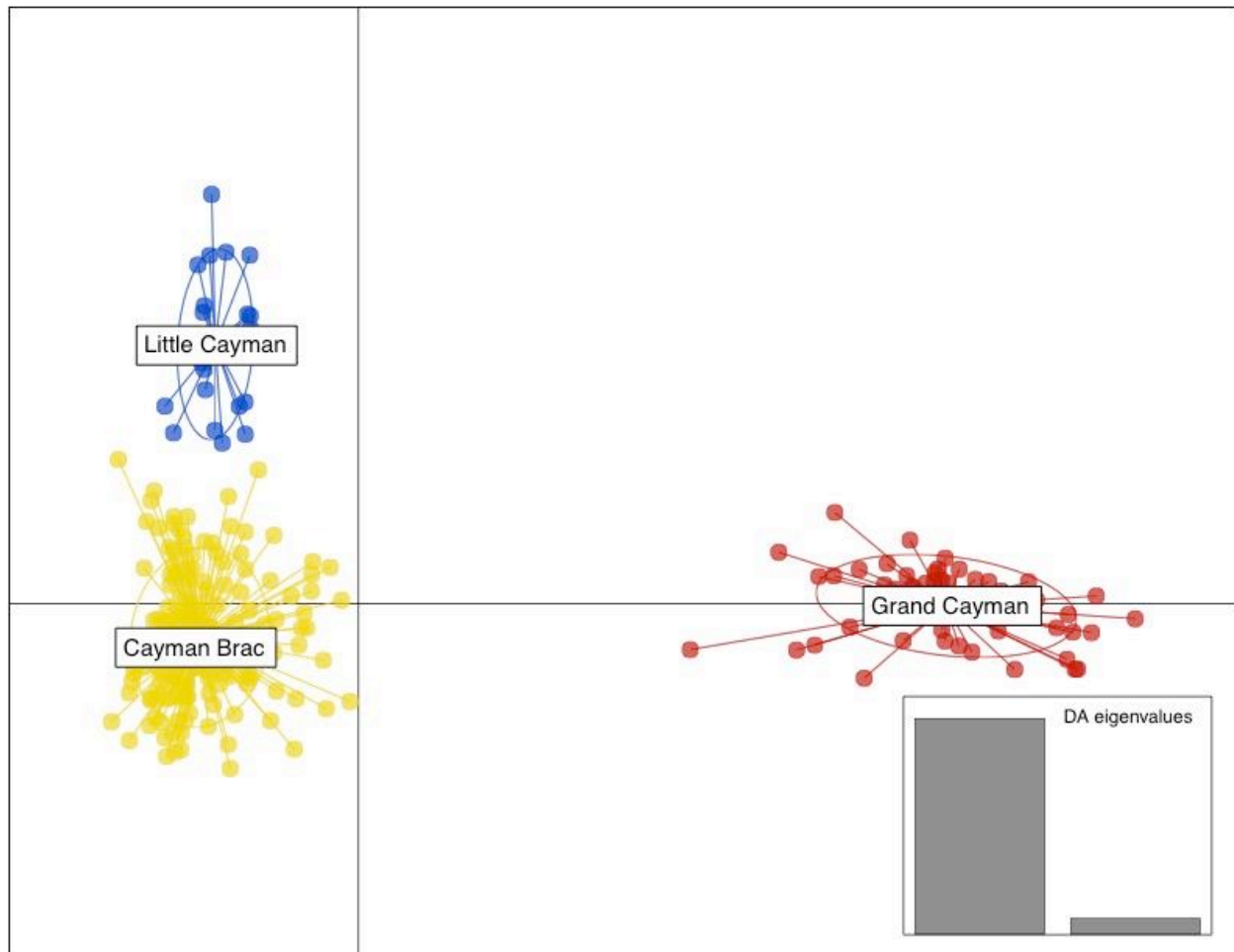


Fig. S3. Maximum likelihood phylogeny showing the relationships among ND2 haplotypes (540 bp) sampled on Cayman Brac, Grand Cayman, Little Cayman, and representative samples from Florida and Cuba that show the invasive history. Little Cayman and Cayman Brac clades are sister to samples from Eastern Cuba (Santiago de Cuba), whereas lizards from Grand Cayman were introduced from non-native populations in south Florida, which originated from multiple sources across Cuba. The star indicates a red-dewlapped lizard (CB008) sampled on Cayman Brac that has an intermediate nuclear genotype and Grand Cayman mtDNA haplotype. Bootstrap values are shown for the major Cayman Island clades.

